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(57) Abstract

Novel polynucleotides and the proteins encoded thereby are disclosed.

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Novel polynucleotides and the proteins encoded thereby are disclosed.

DNA SEQUENCES AND SECRETED PROTEINS ENCODED THEREBY

This application claims priority from application Ser. No. 08/514,014, filed on August 11, 1995, which was converted to provisional application Ser. No. 60/_____ on July 19, 1996.

FIELD OF THE INVENTION

The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins.

BACKGROUND OF THE INVENTION

Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered factor (i.e., partial DNA/amino acid sequence of the factor in the case of hybridization cloning; activity of the factor in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by making available large numbers of DNA/amino acid sequences for factors that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the cell or tissue source in the case of PCR-based techniques. It is to these factors and the polynucleotides encoding them that the present invention is directed.

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 76 to nucleotide 474;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;

(e) a polynucleotide which is an allelic variant of SEQ ID NO:5; and

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 67 to nucleotide 348;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity;

(e) a polynucleotide which is an allelic variant of SEQ ID NO:7; and

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 75 to nucleotide 356;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9 encoding a protein having biological activity;

Compositions comprising a protein biological activity are also disclosed. In preferred embodiments the protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) fragments of the amino acid sequence of SEQ ID NO:2;

(c) the amino acid sequence of SEQ ID NO:4;

(d) fragments of the amino acid sequence of SEQ ID NO:4;

(e) the amino acid sequence of SEQ ID NO:6;

(f) fragments of the amino acid sequence of SEQ ID NO:6;

(g) the amino acid sequence of SEQ ID NO:8;

(h) fragments of the amino acid sequence of SEQ ID NO:8;

(i) the amino acid sequence of SEQ ID NO:12; and

(j) fragments of the amino acid sequence of SEQ ID NO:12;

the protein being substantially free from other mammalian proteins.

Such compositions may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF FIGURES

Fig. 1 is an autoradiograph evidencing the expression of clone J5 in COS cells (indicated by arrows). J5 is processed into multiple bands, with the major band at approximately 58 kD.

Fig. 2 is an autoradiograph evidencing the expression of clone L105 in COS cells (indicated by arrows).

Fig. 3 is an autoradiograph evidencing the expression of clone H174 in COS cells (indicated by arrows).

Fig. 4 is an autoradiograph evidencing the expression of clone B18 in COS cells (indicated by arrows).

identified as "J422_fl", with additional 5' sequence obtained from a second double stranded clone. Clone J422 was isolated from a human activated peripheral blood mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding secreted proteins; therefore, clone J422 does encode a secreted factor. J422 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. However, a FASTA search revealed homology between the J422 protein (in the approximate region of amino acids 34-156 of SEQ ID NO:4) and a number of *Drosophila* leucine-rich repeat (LRR) proteins. Analysis of the full-length J422 sequences revealed that the conserved EGF-domain found in a number of LRR family members was not present in J422. Based upon these homologies, J422 and these homologous proteins are expected to share at least some activities.

The sequence of a polynucleotide encoding another protein of the present invention is set forth in SEQ ID NO:5, with the coding region extending from nucleotides 76 to 474. This polynucleotide has been identified as "clone L105" The amino acid sequence of the protein encoded by clone L105 is set forth in SEQ ID NO:6. Clone L105 was deposited with the American Type Culture Collection on August 11, 1995 and given the accession number ATCC 69883. Clone L105 was isolated from a murine adult thymus library using a trap which selects for nucleotides encoding secreted proteins; therefore, clone L105 does encode a secreted factor. L105 encodes a novel protein: BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. However, a BLASTX search revealed homology between the L105 protein (particularly in the approximate region of amino acids 73-91 of SEQ ID NO:6), various monocyte and other chemoattractant proteins (including without limitation those assigned accession M577441, X71087, X72308, X14768 and M24545) and a chicken (*Gallus gallus*) cytokine (accession L34553). Based upon these homologies, L105 and these homologous proteins are expected to share at least some activities.

The sequence of polynucleotides encoding another protein of the present invention is set forth in SEQ ID NO:7 and SEQ ID NO:9, with the coding regions extending from nucleotides 67 to 348 and nucleotides 75 to 356, respectively. These polynucleotides have been identified as "clone H174-10" and "clone H174-43", respectively (collectively referred to herein as "H174"). The amino acid sequence of

respectively. The bands of protein expressed from the relevant clone are indicated by arrows.

Polynucleotides hybridizing to the polynucleotides of the present invention under stringent conditions and highly stringent conditions are also part of the present invention. As used herein, "highly stringent conditions" include, for example, at least about 0.2xSSC at 65°C; and "stringent conditions" include, for example, at least about 4xSSC at 65°C or at least about 50% formamide, 4xSSC at 42°C. Allelic variants of the polynucleotides of the present invention are also encompassed by the invention.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein of the invention.

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, MA), Pharmacia (Piscataway, NJ) and InVitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used to raise antibodies or to elicit another immune response; as a reagent (including the labelled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these "research tool" utilities are capable of being developed into reagent grade or kit format for commercialization as "research products."

CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION ACTIVITY

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited

Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober

Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

IMMUNE STIMULATING/SUPPRESSING ACTIVITY

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leshmania, malaria and various fungal infections such as candida. Of course, in this regard, a protein of

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al.; J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by denritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimenal Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embyronic differentation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc.., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

TISSUE GENERATION/REGENERATION ACTIVITY

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

It is expected that a protein of the present invention may also exhibit activity for generation of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition of fibrotic scarring to allow normal tissue to regenerate.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

ACTIVIN/INHIBIN ACTIVITY

A protein of the present invention may also exhbit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle

cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

HEMOSTATIC AND THROMBOLYTIC ACTIVITY

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction or stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of ebryonic stem cells in lineages other than hematopoietic lineages; and in the case of enzymes, correcting deficiencies of the enzyme and treating related diseases.

ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being

providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Jacobs, Kenneth
McCoy, John
Kelleher, Kerry
Carlin, McKeough

(ii) TITLE OF INVENTION: DNA SEQUENCES AND SECRETED PROTEINS ENCODED THEREBY

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genetics Institute, Inc. -- Legal Affairs
(B) STREET: 87 CambridgePark Drive
(C) CITY: Cambridge
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02140

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Brown, Scott A.
(B) REGISTRATION NUMBER: 32,724
(C) REFERENCE/DOCKET NUMBER: GI6000

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2209 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 38..1447

Tyr Lys Asn Tyr Asn Glu Asn Leu Thr Leu Ile Arg Ser Phe Val Phe
235 240 245

```
CATGAATTTG TGACTTAGTT CTGCCCTTTG GAGAACAAAA GAAAGCAGTC TTCCATCAAA    1747

TCACCTTAAA ATGCACGGCT AAACTATTCA GAGTTAACAC TCCAGAATTG TTAAATTACA    1807

AGTACTATGC TTTAATGCTT CTTTCATCTT ACTAGTATGG CCTATAAAAA AAATAATACC    1867

ACTTGATGGG TGAAGGCTTT GGCAATAGAA AGAAGAATAG AATTCAGGTT TTATGTTATT    1927

CCTCTGTGTT CACTTCGCCT TGCTCTTGAA AGTGCAGTAT TTTTCTACAT CATGTCGAGA    1987

ATGATTCAAT GTAAATATTT TTCATTTTAT CATGTATATC CTATACACAC ATCTCCTTCA    2047

TCATCATATA TGAAGTTTAT TTTGAGAAGT CTACATTGCT TACATTTTAA TTGAGCCAGC    2107

AAAGAAGGCT TAATGATTTA TTGAACCATA ATGTCAATAA AAACACAACT TTTGAGGCAA    2167

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AA                       2209
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 470 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Leu Arg Gly Ile Ser Gln Leu Pro Ala Val Ala Thr Met Ser Trp
 1               5                  10                  15

Val Leu Leu Pro Val Leu Trp Leu Ile Val Gln Thr Gln Ala Ile Ala
            20                  25                  30

Ile Lys Gln Thr Pro Glu Leu Thr Leu His Glu Ile Val Cys Pro Lys
        35                  40                  45

Lys Leu His Ile Leu His Lys Arg Glu Ile Lys Asn Asn Gln Thr Glu
    50                  55                  60

Lys His Gly Lys Glu Glu Arg Tyr Glu Pro Glu Val Gln Tyr Gln Met
65                  70                  75                  80

Ile Leu Asn Gly Glu Glu Ile Ile Leu Ser Leu Gln Lys Thr Lys His
                85                  90                  95

Leu Leu Gly Pro Asp Tyr Thr Glu Thr Leu Tyr Ser Pro Arg Gly Glu
            100                 105                 110

Glu Ile Thr Thr Lys Pro Glu Asn Met Glu His Cys Tyr Tyr Lys Gly
        115                 120                 125

Asn Ile Leu Asn Glu Lys Asn Ser Val Ala Ser Ile Ser Thr Cys Asp
    130                 135                 140

Gly Leu Arg Gly Tyr Phe Thr His His His Gln Arg Tyr Gln Ile Lys
145                 150                 155                 160

Pro Leu Lys Ser Thr Asp Glu Lys Glu His Ala Val Phe Thr Ser Asn
                165                 170                 175
```

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 52..2034

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATTTCTCAGC TCCAAGCATT AGGTAAACCC ACCAAGCAAT CCTAGCCTGT G ATG GCG      57
                                                          Met Ala
                                                            1

TTT GAC GTC AGC TGC TTC TTT TGG GTG GTG CTG TTT TCT GCC GGC TGT      105
Phe Asp Val Ser Cys Phe Phe Trp Val Val Leu Phe Ser Ala Gly Cys
          5                  10                  15

AAA GTC ATC ACC TCC TGG GAT CAG ATG TGC ATT GAG AAA GAA GCC AAC      153
Lys Val Ile Thr Ser Trp Asp Gln Met Cys Ile Glu Lys Glu Ala Asn
     20                  25                  30

AAA ACA TAT AAC TGT GAA AAT TTA GGT CTC AGT GAA ATC CCT GAC ACT      201
Lys Thr Tyr Asn Cys Glu Asn Leu Gly Leu Ser Glu Ile Pro Asp Thr
 35                  40                  45                  50

CTA CCA AAC ACA ACA GAA TTT TTG GAA TTC AGC TTT AAT TTT TTG CCT      249
Leu Pro Asn Thr Thr Glu Phe Leu Glu Phe Ser Phe Asn Phe Leu Pro
                 55                  60                  65

ACA ATT CAC AAT AGA ACC TTC AGC AGA CTC ATG AAT CTT ACC TTT TTG      297
Thr Ile His Asn Arg Thr Phe Ser Arg Leu Met Asn Leu Thr Phe Leu
            70                  75                  80

GAT TTA ACT AGG TGC CAG ATT AAC TGG ATA CAT GAA GAC ACT TTT CAA      345
Asp Leu Thr Arg Cys Gln Ile Asn Trp Ile His Glu Asp Thr Phe Gln
          85                  90                  95

AGC CAT CAT CAA TTA AGC ACA CTT GTG TTA ACT GGA AAT CCC CTG ATA      393
Ser His His Gln Leu Ser Thr Leu Val Leu Thr Gly Asn Pro Leu Ile
     100                 105                 110

TTC ATG GCA GAA ACA TCG CTT AAT GGG CCC AAG TCA CTG AAG CAT CTT      441
Phe Met Ala Glu Thr Ser Leu Asn Gly Pro Lys Ser Leu Lys His Leu
115                 120                 125                 130

TTC TTA ATC CAA ACG GGA ATA TCC AAT CTC GAG TTT ATT CCA GTG CAC      489
Phe Leu Ile Gln Thr Gly Ile Ser Asn Leu Glu Phe Ile Pro Val His
                135                 140                 145

AAT CTG GAA AAC TTG GAA AGC TTG TAT CTT GGA AGC AAC CAT ATT TCC      537
Asn Leu Glu Asn Leu Glu Ser Leu Tyr Leu Gly Ser Asn His Ile Ser
            150                 155                 160

TCC ATT AAG TTC CCC AAA GAC TTC CCA GCA CGG AAT CTG AAA GTA CTG      585
Ser Ile Lys Phe Pro Lys Asp Phe Pro Ala Arg Asn Leu Lys Val Leu
        165                 170                 175

GAT TTT CAG AAT AAT GCT ATA CAC TAC ATC TCT AGA GAA GAC ATG AGG      633
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AAT GCT CCA CAA AGT CCC TTC CAA AAC CTC CAT TTC CTT CAG GTT CTG      1401
Asn Ala Pro Gln Ser Pro Phe Gln Asn Leu His Phe Leu Gln Val Leu
435                 440                 445                 450

AAT CTC ACT TAC TGC TTC CTT GAT ACC AGC AAT CAG CAT CTT CTA GCA      1449
Asn Leu Thr Tyr Cys Phe Leu Asp Thr Ser Asn Gln His Leu Leu Ala
                455                 460                 465

GGC CTA CCA GTT CTC CGG CAT CTC AAC TTA AAA GGG AAT CAC TTT CAA      1497
Gly Leu Pro Val Leu Arg His Leu Asn Leu Lys Gly Asn His Phe Gln
            470                 475                 480

GAT GGG ACT ATC ACG AAG ACC AAC CTA CTT CAG ACC GTG GGC AGC TTG      1545
Asp Gly Thr Ile Thr Lys Thr Asn Leu Leu Gln Thr Val Gly Ser Leu
        485                 490                 495

GAG GTT CTG ATT TTG TCC TCT TGT GGT CTC CTC TCT ATA GAC CAG CAA      1593
Glu Val Leu Ile Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp Gln Gln
    500                 505                 510

GCA TTC CAC AGC TTG GGA AAA ATG AGC CAT GTA GAC TTA AGC CAC AAC      1641
Ala Phe His Ser Leu Gly Lys Met Ser His Val Asp Leu Ser His Asn
515                 520                 525                 530

AGC CTG ACA TGC GAC AGC ATT GAT TCT CTT AGC CAT CTT AAG GGA ATC      1689
Ser Leu Thr Cys Asp Ser Ile Asp Ser Leu Ser His Leu Lys Gly Ile
                535                 540                 545

TAC CTC AAT CTG GCT GCC AAC AGC ATT AAC ATC ATC TCA CCC CGT CTC      1737
Tyr Leu Asn Leu Ala Ala Asn Ser Ile Asn Ile Ile Ser Pro Arg Leu
            550                 555                 560

CTC CCT ATC TTG TCC CAG CAG AGC ACC ATT AAT TTA AGT CAT AAC CCC      1785
Leu Pro Ile Leu Ser Gln Gln Ser Thr Ile Asn Leu Ser His Asn Pro
        565                 570                 575

CTG GAC TGC ACT TGC TCG AAT ATT CAT TTC TTA ACA TGG TAC AAA GAA      1833
Leu Asp Cys Thr Cys Ser Asn Ile His Phe Leu Thr Trp Tyr Lys Glu
    580                 585                 590

AAC CTG CAC AAA CTT GAA GGC TCG GAG GAG ACC ACG TGT GCA AAC CCG      1881
Asn Leu His Lys Leu Glu Gly Ser Glu Glu Thr Thr Cys Ala Asn Pro
595                 600                 605                 610

CCA TCT CTA AGG GGA GTT AAG CTA TCT GAT GTC AAG CTT TCC TGT GGG      1929
Pro Ser Leu Arg Gly Val Lys Leu Ser Asp Val Lys Leu Ser Cys Gly
                615                 620                 625

ATT ACA GCC ATA GGC ATT TTC TTT CTC ATA GTA TTT CTA TTA TTG TTG      1977
Ile Thr Ala Ile Gly Ile Phe Phe Leu Ile Val Phe Leu Leu Leu Leu
            630                 635                 640

GCT ATT CTG CTA TTT TTT GCA GTT AAA TAC CTT CTC AGG TGG AAA TAC      2025
Ala Ile Leu Leu Phe Phe Ala Val Lys Tyr Leu Leu Arg Trp Lys Tyr
        645                 650                 655

CAA CAC ATT TAGTGCTGAA GGTTTCCAGA GAAAGCAAAT AAGTGTGCTT              2074
Gln His Ile
    660

AGCAAAATTG CTCTAAGTGA AAGAACTGTC ATCTGCTGGT GACCAGACCA GACTTTTCAG    2134

ATTGCTTCCT GGAACTGGGC AGGGACTCAC TGTGCTTTTC TGAGCTTCTT ACTCCTGTGA    2194
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195 200 205

Asn Asn Val Lys Gly Ile Glu Leu Gly Ala Phe Asp Ser Thr Val Phe
210 215 220

Gln Ser Leu Asn Phe Gly Gly Thr Pro Asn Leu Ser Val Ile Phe Asn
225 230 235 240

Gly Leu Gln Asn Ser Thr Thr Gln Ser Leu Trp Leu Gly Thr Phe Glu
245 250 255

Asp Ile Asp Asp Glu Asp Ile Ser Ser Ala Met Leu Lys Gly Leu Cys
260 265 270

Glu Met Ser Val Glu Ser Leu Asn Leu Gln Glu His Arg Phe Ser Asp
275 280 285

Ile Ser Ser Thr Thr Phe Gln Cys Phe Thr Gln Leu Gln Glu Leu Asp
290 295 300

Leu Thr Ala Thr His Leu Lys Gly Leu Pro Ser Gly Met Lys Gly Leu
305 310 315 320

Asn Leu Leu Lys Lys Leu Val Leu Ser Val Asn His Phe Asp Gln Leu
325 330 335

Cys Gln Ile Ser Ala Ala Asn Phe Pro Ser Leu Thr His Leu Tyr Ile
340 345 350

Arg Gly Asn Val Lys Lys Leu His Leu Gly Val Gly Cys Leu Glu Lys
355 360 365

Leu Gly Asn Leu Gln Thr Leu Asp Leu Ser His Asn Asp Ile Glu Ala
370 375 380

Ser Asp Cys Cys Ser Leu Gln Leu Lys Asn Leu Ser His Leu Gln Thr
385 390 395 400

Leu Asn Leu Ser His Asn Glu Pro Leu Gly Leu Gln Ser Gln Ala Phe
405 410 415

Lys Glu Cys Pro Gln Leu Glu Leu Leu Asp Leu Ala Phe Thr Arg Leu
420 425 430

His Ile Asn Ala Pro Gln Ser Pro Phe Gln Asn Leu His Phe Leu Gln
435 440 445

Val Leu Asn Leu Thr Tyr Cys Phe Leu Asp Thr Ser Asn Gln His Leu
450 455 460

Leu Ala Gly Leu Pro Val Leu Arg His Leu Asn Leu Lys Gly Asn His
465 470 475 480

Phe Gln Asp Gly Thr Ile Thr Lys Thr Asn Leu Leu Gln Thr Val Gly
485 490 495

Ser Leu Glu Val Leu Ile Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp
500 505 510

Gln Gln Ala Phe His Ser Leu Gly Lys Met Ser His Val Asp Leu Ser
515 520 525

His Asn Ser Leu Thr Cys Asp Ser Ile Asp Ser Leu Ser His Leu Lys

```
GCA ATC CTG TTC TCA CCC CGG AAG CAC TCT AAG CCT GAG CTA TGT GCA      303
Ala Ile Leu Phe Ser Pro Arg Lys His Ser Lys Pro Glu Leu Cys Ala
                65                  70                  75

AAC CCT GAG GAA GGC TGG GTG CAG AAC CTG ATG CGC CGC CTG GAC CAG      351
Asn Pro Glu Glu Gly Trp Val Gln Asn Leu Met Arg Arg Leu Asp Gln
            80                  85                  90

CCT CCA GCC CCA GGG AAA CAA AGC CCC GGC TGC AGG AAG AAC CGG GGA      399
Pro Pro Ala Pro Gly Lys Gln Ser Pro Gly Cys Arg Lys Asn Arg Gly
        95                  100                 105

ACC TCT AAG TCT GGA AAG AAA GGA AAG GGC TCC AAG GGC TGC AAG AGA      447
Thr Ser Lys Ser Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg
    110                 115                 120

ACT GAA CAG ACA CAG CCC TCA AGA GGA TAGCCCAGTA GCCCGCCTGG            494
Thr Glu Gln Thr Gln Pro Ser Arg Gly
125                 130

AGCCCAGGAG ATCCCCCACG AACTTCAAGC TGGGTGGTTC ACGGTCCAAC TCACAGGCAA    554

AGAGGGAGCT AGAAAACAGA CTCAGGAGCC GCTA                               588
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Gln Met Met Thr Leu Ser Leu Leu Ser Leu Val Leu Ala Leu
  1               5                  10                  15

Cys Ile Pro Trp Thr Gln Gly Ser Asp Gly Gly Gly Gln Asp Cys Cys
            20                  25                  30

Leu Lys Tyr Ser Gln Lys Lys Ile Pro Tyr Ser Ile Val Arg Gly Tyr
        35                  40                  45

Arg Lys Gln Glu Pro Ser Leu Gly Cys Pro Ile Pro Ala Ile Leu Phe
    50                  55                  60

Ser Pro Arg Lys His Ser Lys Pro Glu Leu Cys Ala Asn Pro Glu Glu
65                  70                  75                  80

Gly Trp Val Gln Asn Leu Met Arg Arg Leu Asp Gln Pro Pro Ala Pro
                85                  90                  95

Gly Lys Gln Ser Pro Gly Cys Arg Lys Asn Arg Gly Thr Ser Lys Ser
            100                 105                 110

Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg Thr Glu Gln Thr
        115                 120                 125

Gln Pro Ser Arg Gly
    130
```

AAGCATTCCT CAAACATTTA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 948

AAAAAAAAAA AAAAAAAA 966

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val Ile Leu Cys Ala
1 5 10 15

Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly Arg Cys Leu Cys
20 25 30

Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp Ile Glu Lys Ala
35 40 45

Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile Glu Val Ile Ile
50 55 60

Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn Pro Lys Ser Lys
65 70 75 80

Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys Asn Phe
85 90

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 75..356

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTCTACTCCT TCCAAGAAGA GCAGCAAAGC TGAAGTAGCA GCAACAGCAC CAGCAGCAAC 60

AGCAAAAAAC AAAC ATG AGT GTG AAG GGC ATG GCT ATA GCC TTG GCT GTG 110
Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val
1 5 10

ATA TTG TGT GCT ACA GTT GTT CAA GGC TTC CCC ATG TTC AAA AGA GGA 158
Ile Leu Cys Ala Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly
15 20 25

```
Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val Ile Leu Cys Ala
 1               5                  10                  15

Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly Arg Cys Leu Cys
            20                  25                  30

Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp Ile Glu Lys Ala
        35                  40                  45

Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile Glu Val Ile Ile
    50                  55                  60

Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn Pro Lys Ser Lys
65                  70                  75                  80

Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys Asn Phe
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 813 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 86..544

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GGGAAGATAC ATTCACAGAA AGAGCTTCCT GCACAAAGTA AGCCACCAGC GCAACATGAC      60

AGTGAAGACC CTGCATGGCC CAGCC ATG GTC AAG TAC TTG CTG CTG TCG ATA      112
                            Met Val Lys Tyr Leu Leu Leu Ser Ile
                              1               5

TTG GGG CTT GCC TTT CTG AGT GAG GCG GCA GCT CGG AAA ATC CCC AAA      160
Leu Gly Leu Ala Phe Leu Ser Glu Ala Ala Arg Lys Ile Pro Lys
 10                  15                  20                  25

GTA GGA CAT ACT TTT TTC CAA AAG CCT GAG AGT TGC CCG CCT GTG CCA      208
Val Gly His Thr Phe Phe Gln Lys Pro Glu Ser Cys Pro Pro Val Pro
                30                  35                  40

GGA GGT AGT ATG AAG CTT GAC ATT GGC ATC ATC AAT GAA AAC CAG CGC      256
Gly Gly Ser Met Lys Leu Asp Ile Gly Ile Ile Asn Glu Asn Gln Arg
            45                  50                  55

GTT TCC ATG TCA CGT AAC ATC GAG AGC CGC TCC ACC TCC CCC TGG AAT      304
Val Ser Met Ser Arg Asn Ile Glu Ser Arg Ser Thr Ser Pro Trp Asn
        60                  65                  70

TAC ACT GTC ACT TGG GAC CCC AAC CGG TAC CCC TCG GAA GTT GTA CAG      352
Tyr Thr Val Thr Trp Asp Pro Asn Arg Tyr Pro Ser Glu Val Val Gln
    75                  80                  85
```

130 135 140

Val Thr Pro Val Ile His His Val Gln
145 150

(a) the amino acid sequence of SEQ ID NO:2; and

(b) fragments of the amino acid sequence of SEQ ID NO:2;

the protein being substantially free from other mammalian proteins.

8. The composition of claim 7, further comprising a pharmaceutically acceptable carrier.

9. A composition comprising an antibody which specifically reacts with the protein of claim 7.

10. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 8.

11. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 52 to nucleotide 2034;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;

(e) a polynucleotide which is an allelic variant of SEQ ID NO:4; and

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

12. A composition of claim 11 wherein said polynucleotide is operably linked to an expression control sequence.

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;

(e) a polynucleotide which is an allelic variant of SEQ ID NO:5; and

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

22. A composition of claim 21 wherein said polynucleotide is operably linked to an expression control sequence.

23. A host cell transformed with a composition of claim 22.

24. The host cell of claim 23, wherein said cell is a mammalian cell.

25. A process for producing a protein, which comprises:

(a) growing a culture of the host cell of claim 23 in a suitable culture medium; and

(b) purifying the protein from the culture

26. A protein produced according to the process of claim 25.

27. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:6; and

(b) fragments of the amino acid sequence of SEQ ID NO:6;

the protein being substantially free from other mammalian proteins.

35. A process for producing a protein, which comprises:

(a) growing a culture of the host cell of claim 33 in a suitable culture medium; and

(b) purifying the protein from the culture

36. A protein produced according to the process of claim 35.

37. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:8; and

(b) fragments of the amino acid sequence of SEQ ID NO:8;

the protein being substantially free from other mammalian proteins.

38. The composition of claim 37, further comprising a pharmaceutically acceptable carrier.

39. A composition comprising an antibody which specifically reacts with the protein of claim 37.

40. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 38.

41. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 75 to nucleotide 356;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

50. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 48.

51. A composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 86 to nucleotide 544;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11 encoding a protein having biological activity;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;

(e) a polynucleotide which is an allelic variant of SEQ ID NO:11; and

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

52. A composition of claim 51 wherein said polynucleotide is operably linked to an expression control sequence.

53. A host cell transformed with a composition of claim 52.

54. The host cell of claim 53, wherein said cell is a mammalian cell.

55. A process for producing a protein, which comprises:

(a) growing a culture of the host cell of claim 53 in a suitable culture medium; and

(b) purifying the protein from the culture

56. A protein produced according to the process of claim 55.